

# Release of Metal Impurities from Carbon Nanomaterials Influences Aquatic Toxicity

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Received September 3, 2008. Revised manuscript received March 24, 2009. Accepted March 25, 2009.

Few studies have considered the environmental impacts of impurities and byproducts associated with low-efficiency nanomanufacturing processes. Here, we study the composition and aquatic toxicity of low-purity, as-produced fullerenes (C<sub>60</sub>) and metallofullerene waste solids, both of which were generated via arc-discharge synthesis. Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX) and inductively coupled plasma mass spectroscopy (ICP-MS) were used to characterize the metals composition of the solid test materials and of aqueous leachates prepared by mixing test materials with waters of varying pH, hardness, and salinity. The aquatic toxicity of the leachates was determined using U.S. Environmental Protection Agency recommended aquatic bioassay protocols with two standard test organisms—*Pimephales promelas* and *Ceriodaphnia dubia*. Results indicated that metals associated with the solid test materials became mobilized in our test system upon interaction with waters of circumneutral pH and reached concentrations sufficient to induce toxicity in both test species. Acute (48 h) LC<sub>50</sub> values for *P. promelas* and *C. dubia* exposed to leachates prepared from metallofullerene waste solids were 54 and 5% (as % leachate in diluent), respectively. Toxicity was eliminated after adding the chelator EDTA to the leachates, implicating divalent transition metals as the toxicity source. Our results demonstrate the aquatic toxicity of metals mobilized from products and byproducts of nanomanufacturing, and they emphasize the need for a global review of nanomanufacturing wastes and low-purity products.

## Introduction

Estimates suggest that by 2015, nanotechnology will have a trillion-plus dollar global economic impact (1, 2). Already, commercial applications of nanotechnology are increasing,

varying from use in next-generation pharmaceuticals to high-efficiency photovoltaics (3–9). Growing demand for products containing engineered nanomaterials has prompted manufacturers to scale up operations to offer materials at increasingly competitive prices. As the scale of nanomanufacturing expands and material usage increases, so too will the likelihood that engineered nanomaterials and associated waste byproducts will be dispersed in the environment (10). Studies have suggested that some engineered nanomaterials pose environmental health and safety (EHS) risks. Griffith et al. (11) reported that copper nanoparticles were more acutely toxic to zebrafish than could be explained by dissolution alone. Derfus et al. (12) reported that oxidation of CdSe quantum dots resulted in leaching of Cd<sup>2+</sup> ions, which induced cytotoxicity to hepatocytes in vitro. A number of other studies have reported that carbon nanomaterials were toxic in mammalian systems (13–17). At least one study has reported enhanced mobility of engineered nanomaterials in simulated groundwater systems (18). These findings, coupled with the unprecedented growth of the nanotechnology industry, have spurred research into the human and environmental health and safety implications of engineered nanomaterials.

The majority of studies published on nanotechnology EHS have focused on the final nanoscale products, and have not considered the potential impacts of impurities and synthesis byproducts (e.g., residual metals, organic solvents) on the range of nanomaterial effects reported in the literature. Although actual nanomanufacturing efficiency data are difficult to obtain (19), waste byproducts can comprise approximately 40 to >99.9% of the total production output (on a mass basis) for some of the least efficient nanomanufacturing processes (20). These byproducts may take the form of impurities in low-grade products, or as components of mixed solid-waste streams. As the scale of nanomanufacturing reaches commercial proportions, management of these undesired process outputs may become more problematic, particularly if production efficiencies remain low. Thus, coupled with the immediate need to investigate potential effects of released engineered nanomaterials in environmental systems, there exists a pressing need to study the composition and potential hazards posed by the mixed waste streams and crude product extracts that are generated during some nanomanufacturing processes (21).

Metals-laden wastes are of particular concern given the known toxicological effects of metals on living systems (22–26) and the widespread use of metals in the synthesis of nanoscale products such as metal-containing fullerenes (27) and carbon nanotubes (28). Previous studies have indicated the importance of metals in the synthesis and manufacturing of a number of different classes of engineered nanomaterials, as well as the potential of these metals to remain associated with the final nanoscale products and waste streams. Stevenson et al. (29) and Tian et al. (30) noted enhanced yields of specialized fullerene species and carbon nanotubes, respectively, upon the addition of Cu metal powder to the initial reactant mixture. Methods for the preparation of semiconducting quantum dots describe reliance on the decomposition of metal complexes (e.g., Cd, Pb) in the presence of acids and metal precursors (31). Pumera (32) recently noted that residual Fe, Ni, Co, and Mo impurities, which are used as catalysts during production and growth of carbon nanotubes by chemical vapor deposition (CVD), remain in samples of multi- and single-walled carbon nanotubes even after acid-washing at high temperatures. Given the frequent usage of metals as feedstocks for many

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Report Documentation Page			Form Approved OMB No. 0704-0188		
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1. REPORT DATE <b>2009</b>	2. REPORT TYPE		3. DATES COVERED <b>00-00-2009 to 00-00-2009</b>		
4. TITLE AND SUBTITLE <b>Release Of Metal Impurities From Carbon Nanomaterials Influences Aquatic Toxicity</b>			5a. CONTRACT NUMBER		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S)			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>U.S. Army Engineer Research and Development Center,3909 Halls Ferry Road,Vicksburg,MS,39180</b>			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>					
13. SUPPLEMENTARY NOTES <b>Environmental Science &amp; Technology 43, No 11,Government or Federal Purpose Rights License.</b>					
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15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>Same as Report (SAR)</b>	18. NUMBER OF PAGES <b>7</b>	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			



nanoscale synthesis procedures, it is reasonable to assume that these metals will also be major constituents of nanomanufacturing outputs, as either impurities in products or as components of solid waste streams.

The current study addresses whether feedstock-associated metals pose potential risks to aquatic receptors. The study was intended to screen whether mobilized impurities from nanomaterials and nanomaterial byproducts may be of potential concern for environmental regulators and for researchers attempting to address toxicity due to nanoparticles alone. Specific modeling of impurity concentrations that may partition into the environment was beyond the scope of this study. We investigated the metals composition of two different nanomanufacturing outputs: a commercially available, as-produced fullerene product, and two distinct samples of metal-containing fullerene (i.e., metallofullerene) waste byproduct. We then determined whether feedstock metals associated with the production of these materials can become mobilized and reach toxic concentrations in simulated aquatic systems.

## Experimental Section

**Test Materials.** Three test materials were selected for study based on the established association of metals with their production as well as the existing use of these materials in research and commerce. All materials were generated using the Krätschmer–Huffman arc method. The first material, a commercially available, as-produced  $C_{60}$  fullerene soot (catalog no. 572497), was obtained from Sigma-Aldrich (St. Louis, MO). The final product is reported to contain greater than 7% fullerenes by weight; of that fraction, typical fullerene composition is as follows:  $C_{60}$  (76%),  $C_{70}$  (22%), and higher-order fullerenes (2%). Information on metals composition was not provided by the distributor. The second and third materials, mixed solid waste composites resulting from the manufacture of gadolinium encapsulate ( $Gd_3N@C_{80}$ ) endohedral metallofullerenes, were obtained from an unnamed industrial source and a university research laboratory. Endohedral metallofullerenes are carbonaceous fullerenes encapsulating metal atoms bound to a nitrogen core. The synthesis of these materials follows the trimetallic nitride template process (33). The final product of this synthesis procedure is extracted from an enriched soot by high-performance liquid chromatography (33, 27). The yield of finalized nanomaterial product is low, resulting in relatively large amounts of byproduct material and future emphasis on greater efficiency (20, 34). Additional details are provided in the Supporting Information.

**Preparation of Leachates.** Experiments were performed to determine whether metallic impurities present in the test materials could leach from solid material into waters having characteristics representative of natural surface waters (i.e., similar pH and hardness). Leachates were prepared using a method adapted from the toxicity characteristic leaching procedure (TCLP, U.S. EPA test method 1311). The procedure was modified as described below to accommodate reduced mass of materials, shortened contact time, and stream-lined analysis. Briefly, one gram of each of the materials was added to one-liter sample bottles (Nalgene, Rochester, NY) filled with reconstituted waters of varying chemistry (see the Supporting Information for measured water quality parameters of the reconstituted waters). Moderately hard reconstituted water (MHRW) and hard reconstituted water (HRW) were formulated in accordance with U.S. EPA standard methods (35). Estuarine (5‰) and marine (30‰) waters were formulated using Instant Ocean sea salt (Aquarium Systems, Mentor, OH), according to manufacturer specifications. Estuarine and marine waters were used only for chemical analysis; only freshwaters were used in aquatic bioassays. Upon addition to the reconstituted test waters, the solids

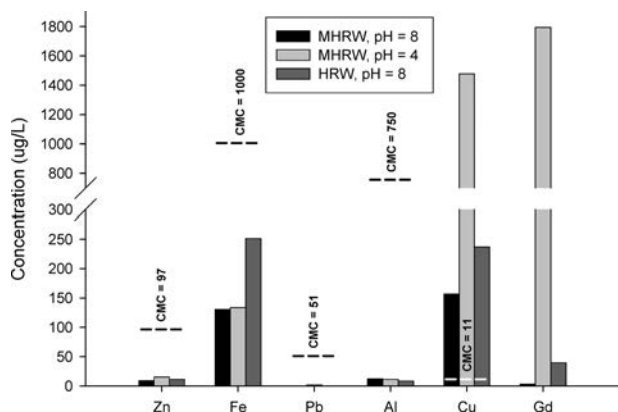
were hydrophobic and did not readily dissolve or disperse. To maintain environmental relevance, however, solids were dispersed into waters without the use of solvents or other preparatory methods (e.g., sonication, addition of surfactants) that enhance material dissolution or dispersion. The bottles containing the solid fractions were placed on an orbital shaker at a speed of 100 RPM for one hour and allowed to settle overnight. Following settling, the bottles were centrifuged at speeds up to 4200g. The supernatant was filtered using 0.45  $\mu$ m Millipore paper filters (HAWP 047 00, Millipore Corporation, Bedford, MA). The resulting clear filtrate was collected and used for subsequent metals analysis and aquatic bioassays with U.S. EPA standard test organisms; the leached solid fractions were discarded and not used in bioassays.

**Determination of Metals in Leachates and Solids.** The bulk chemical composition of as-produced  $C_{60}$  and metallofullerene byproduct materials was measured using a scanning electron microscope outfitted with a Fisons-Kevex 771 energy dispersive X-ray spectroscopy (EDX) analyzer (Quantax System; Bruker AXS, Ewing, NJ). Leachates were analyzed by ICP-MS for feedstock metals associated with the arc-discharge method. The following is a list of metals analyzed by ICP-MS: Al, Be, Cd, Ce, Co, Cr, Cu, Fe, Gd, Mo, Nd, Ni, Pb, Pr, Sb, Sm, Sn, Tb, Th, Ti, Tl, Tm, V, Y, Yb, and Zn. Metals concentrations were determined in the aqueous leachates after acid digestion (EPA Method 3010A), using a Perkin-Elmer Elan 6000 ICP-MS following EPA Method 6020. Detection limits were <1 and 50  $\mu$ g/kg for the aqueous phase and solid phase metals, respectively. Refer to the Supporting Information for additional details on ICP-MS.

**Bioassays with Leachates and Solids.** The acute water column toxicity of as-produced  $C_{60}$  and metallofullerene waste leachates prepared from freshwaters (MHRW and HRW) of circum-neutral pH was assessed at  $25 \pm 1^\circ\text{C}$  using 48 h test methods for *Ceriodaphnia dubia* and *Pimephales promelas* (35). These organisms were selected for their demonstrated sensitivity to metals (23), and their frequent use in ecotoxicological evaluations of surface waters, industrial effluents, and a broad spectrum of chemicals used in commerce. A 10 day sediment toxicity test for survival and growth of the freshwater amphipod, *Hyalella azteca*, exposed to sediments amended with a second solid-phase metallofullerene waste was also conducted. Details on aquatic and sediment bioassay methods and statistical analysis are provided in the Supporting Information. Lethal concentration values inducing 50% mortality (LC50) were calculated using the trimmed Spearman–Karber Method.

**EDTA Toxicity Reduction Experiment.** Many of the cationic metals (e.g., Cd, Cu, Ni, Pb, Zn) associated with carbonaceous nanomanufacturing can be chelated by ethylenediaminetetraacetic acid (EDTA) and made less bioavailable to test organisms (36); thus, we tested the hypothesis that any metals-induced toxicity observed in the aquatic bioassays with diluted leachates could be eliminated when treated with EDTA. Separate aquatic bioassays were performed using 100% as-produced  $C_{60}$  and metallofullerene waste leachates treated with EDTA 60 min prior to the addition of test organisms (EDTA was obtained from Sigma Chemical Co., CAS No. 6381-92-6, 99.5% purity). EDTA was added to the leachates at 0.0024–479 mM concentrations, and therefore was present in excess to the Ca (0.3–0.7 mM) and Mg (0.5–1.0 mM), leaving the excess available for binding to the toxic metals. Additionally, the formation constants for Ca and Mg-EDTA complexes are  $4.9 \times 10^{10}$  and  $6.2 \times 10^8$ , respectively, which are much lower than the formation constants of copper and gadolinium EDTA formation constants of  $6.3 \times 10^{18}$  and  $2.3 \times 10^{17}$ , respectively. This indicates that even if EDTA were not present in excess, the binding of copper and gadolinium would still be preferred relative to calcium and magnesium. Leachates treated with EDTA were





**FIGURE 1. Metals concentrations for the subset of freshwater leachates. Metals not shown were below the detection limits ( $<1 \mu\text{g/L}$ ). Where available, acute water quality criteria for specific metals are provided as the criterion maximum concentration (CMC) designated by the U.S. EPA. The CMC for Cu is  $11 \mu\text{g/L}$ . Metals concentrations for leachates prepared at various ionic strengths as well as leachates prepared from as-produced  $\text{C}_{60}$  leachates in MHRW are available in the Supporting Information.**

tested in parallel with the dilution assays described in the previous section (refer to the Supporting Information for methods describing leachate water chemistry).

## Results and Discussion

**Mobilization of Metals.** Results of qualitative SEM-EDX measurements (Supporting Information) indicated that the surfaces of the as-produced  $\text{C}_{60}$  product and the metallofullerene wastes were primarily comprised of carbon and oxygen-containing groups. Three process-associated metals, Fe, Gd, and Cu, were detected on the surfaces of the metallofullerene wastes (Gd was the target encapsulate metal, whereas Cu and Fe were used as catalysts). This was not surprising given previous studies have documented the strong association between metals and oxidized carbon surfaces (37, 38). Analysis by ICP-MS of the leachate water (detection limit =  $1 \mu\text{g/L}$ ) from the as-produced  $\text{C}_{60}$  and metallofullerene waste solids indicated some metals mobilized from solid particulate surfaces into aqueous solution (Figure 1). In addition to Fe, Gd, and Cu, metallofullerene waste leachates contained Al, Pb, and Zn at concentrations ranging from 2–15 and 1–61  $\mu\text{g/L}$  for leachates prepared from fresh and estuarine waters, respectively. As-produced  $\text{C}_{60}$  leachates (which were prepared in MHRW only) contained a range of metals, but at much lower concentrations than observed in the metallofullerene waste leachates (see the Supporting Information). One metal, Ba, and one nonmetal, B, were present at high concentrations in the  $\text{C}_{60}$  leachate. The pH 4 metallofullerene waste leachate had the greatest concentrations of dissolved metals (Figure 1); Cu and Gd concentrations increased from 157 and 0.09 to 1478 and 1794  $\mu\text{g/L}$ , respectively, when pH was decreased from 8 to 4. This inverse relationship between pH and dissolved metals was expected given that metals are more soluble in acidic solution (39). Higher concentrations of metals were observed in the higher ionic strength waters relative to freshwater (Supporting Information), a finding potentially explained by increased competition for binding sites between metal cations present in the waste leachates and those released by the dissolution of salts used to prepare the high ionic strength dilution media. Calcium and magnesium are present at factors of 90 and 77 greater than the copper and 4000 greater than gadolinium, respectively, and therefore could compete with these metals for sorption sites on the soot material in a way analogous to soil cation exchange capacity determinations in which

sorption sites are loaded with sodium ions that are then displaced and measured by an excess of ammonium.

**Leachate Toxicity.** Leachates prepared from as-produced  $\text{C}_{60}$  and metallofullerene waste in MHRW were acutely toxic to both test species (Table 1). Metallofullerene waste leachates were notably more toxic than those prepared from as-produced  $\text{C}_{60}$ , and induced complete mortality in *C. dubia* at concentrations as low as 6% leachate (lowest observable effect concentration; LOEC). In contrast, for leachates prepared from as-produced  $\text{C}_{60}$ , only the 100% leachate concentration induced complete mortality of test organisms. A similar trend was evident for *P. promelas*; an LOEC of 50% was estimated for metallofullerene waste leachate whereas an LOEC for as-produced  $\text{C}_{60}$  could not be determined due to insufficient mortality. The greater toxicity of metallofullerene waste leachates relative to leachates prepared from as-produced  $\text{C}_{60}$  may be at least partially attributable to the greater fraction of metals observed in the waste leachate. For example, at comparable pH and hardness, the Cu concentration present in metallofullerene waste leachate was seven times greater than that measured in as-produced  $\text{C}_{60}$  leachate. Relative to *C. dubia*, *P. promelas* was more tolerant to both the as-produced  $\text{C}_{60}$  and metallofullerene waste leachates; mean survivorship for *P. promelas* was  $10 \pm 8\%$  in the 100% metallofullerene waste leachate compared to 0% (complete mortality) for *C. dubia*. The 48 h toxicity reference values are supplied in Table 1.

**Toxicity Elimination by EDTA Chelation and Water Hardness.** Treating both the as-produced  $\text{C}_{60}$  and metallofullerene waste leachates with EDTA eliminated the toxicity observed in untreated leachates, thus implicating metal exposure as the underlying cause of toxicity. The survival of both species was indistinguishable from the control when the 100% test water was treated with EDTA (Figure 2). Previous studies have shown that EDTA chelates metals such as Gd (40), Cd, Cu, Ni, Pb, and Zn, making them less bioavailable and thus less toxic; however, EDTA is less effective at reducing toxicity caused by Fe, Cr, and Al (36), which were also detected in the test water. As shown in Figures 2 and 3, Cu was present in both as-produced  $\text{C}_{60}$  and metallofullerene test waters. Further, Cu was the only metal to exceed its criterion maximum concentration (CMC) of  $11 \mu\text{g/L}$  (hardness = 80  $\text{mg/L}$  as  $\text{CaCO}_3$ ) as set forth by the U.S. EPA National Recommended Water Quality Criteria (41) and toxicity reference values reported in the literature (42). Collectively, these results suggest that the presence of metals, particularly Cu, in leachate water most likely contributed to the toxicity observed in the aquatic bioassays. A more comprehensive TCLP procedure (TCLP, U.S. EPA Test Method 1311) followed by a toxicity identification and reduction evaluation (TIE/TRE) could be employed by generators of complex nanomanufacturing waste-streams to identify specific toxicants in liquid, solid, or multiphasic wastes. Given the time and resource intensiveness of these procedures, however, they would likely need to be performed under a narrow range of environmental conditions (e.g., pH, ionic strength, natural organic matter) specifically suited to the site(s) where potential leaching of nanomanufacturing wastes would be of concern (e.g., landfill, settling pond).

Water hardness also ameliorated some toxicity. Although complete mortality was observed in the 100% leachate treatment created in HRW, *C. dubia* was significantly more tolerant in this higher water hardness leachate with a 48 h  $\text{LC}_{50}$  of 11 (10–13%); this difference increased once the  $\text{LC}_{50}$  values in the MHRW and HRW were normalized to concentrations of Cu metal (Figure 3), and the increase in tolerance was consistent with findings reported in the literature for *C. dubia* exposed to metals at increasing water hardness (24).

**TABLE 1. Toxicity End Points (48 h) for *Ceriodaphnia dubia* and *Pimephales promelas* Expressed As a Percentage of the Leachate Water for the Metallofullerene Waste (a) and As-Produced C<sub>60</sub> (b)<sup>a</sup>**

test organism	48 h end point	toxicity reference value (% leachate)	EDTA metals (μg/L)			other metals (μg/L)	
			Cu	Gd	Zn	Al	Fe
<i>Ceriodaphnia dubia</i>	LC <sub>50</sub> (95%CI)	5 (14–15)	8	<1	<1	<1	7
	LOEC	6	9	<1	<1	<1	8
	NOEC	3	5	<1	<1	<1	4
<i>Pimephales promelas</i>	LC <sub>50</sub>	54 (46–63)	85	2	5	7	70
	LOEC	50	79	2	5	6	65
	NOEC	25	39	<1	2	3	33

test organism	48 h end point	toxicity reference value (% leachate)	EDTA metals (μg/L)			Other metals (μg/L)				
			Cu	Ni	Zn	Al	Ba	Cr	Mn	Sr
<i>Ceriodaphnia dubia</i>	LC <sub>50</sub> (95%CI)	60 (52–68)	16	<1	4	1	60	1	3	7
	LOEC	100	26	1	7	2	100	2	5	11
	NOEC	50	13	<1	3	1	50	1	2	6
<i>Pimephales promelas</i>	LC <sub>50</sub>	>100	26	1	7	2	100	2	5	11
	LOEC	>100	26	1	7	2	100	2	5	11
	NOEC	100	26	1	7	2	100	2	5	11

<sup>a</sup> Concentrations for metals that are and are not bound by EDTA are presented based on the percent leachate at each toxicity reference value. Numbers in parentheses represent the 95% confidence interval. Metals not reported were <1 μg/L.

**Significance to Environment and Bioassay Conduct.** Our results indicate that feedstock metals, some of which are known to be toxic in environmental systems, can be found in low-purity fullerene products and mixed nanomanufacturing waste streams. Under pH conditions typical of surface waters and natural rainfall, these metals may partition from solid phases into soluble forms that are toxic to aquatic organisms. Aquatic bioassays using metals-sensitive test organisms exposed to EDTA treated/nontreated test waters lend further evidence to our claim that the mobilization of metals from as-produced C<sub>60</sub> and metallofullerene wastes plays a significant role in the toxicity of the materials and leachates evaluated here. This has significant implications to the environmental effects of the leaching of waste products and to the toxicity testing of finalized but impure nanomaterial products. Tests of a second metallofullerene solid phase waste amended into a freshwater sediment, the ultimate repository for this hydrophobic material, suggests toxicological implications of this solid phase at higher concentrations due to reduced contaminant bioavailability (see the Supporting Information for full data reporting).

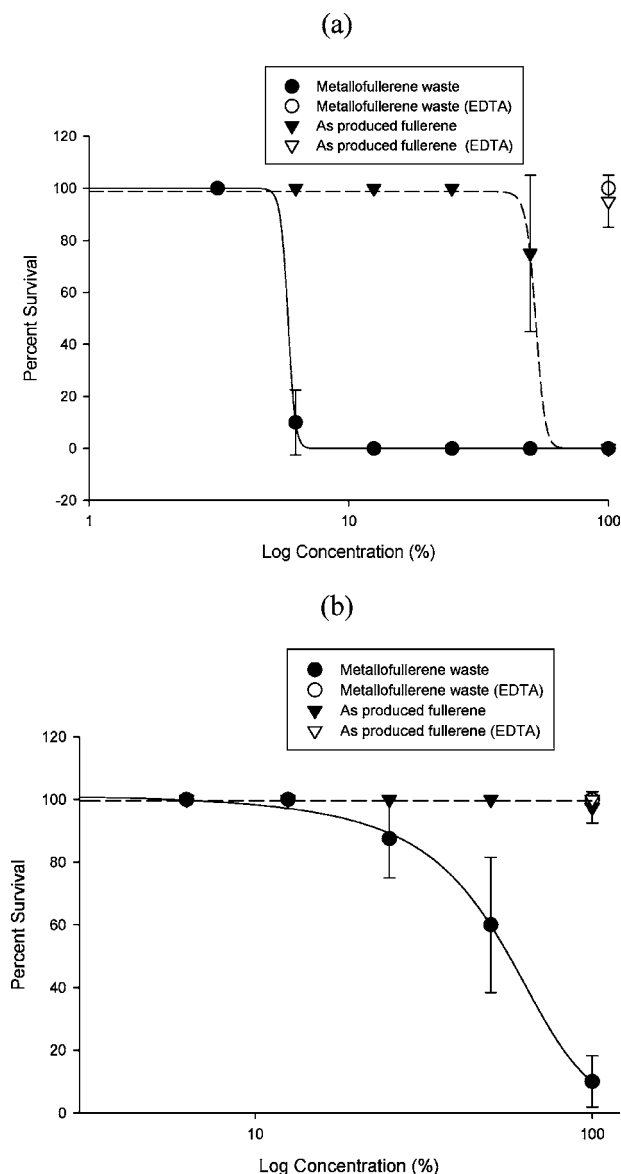
At present, it is unclear whether the metals concentrations in our laboratory studies are likely to occur in the environment, particularly when considered alongside naturally occurring metals complexation reactions. However, previous experience with leachates from landfills suggests that potential exists for high concentrations of metals (or other contaminants) to be observed in areas where the mass of waste materials may be concentrated (e.g., a fly ash landfill or finishing pond), contact times may be increased (years instead of days), and site-specific conditions may be more conducive to mobilization (i.e., pH, redox, microbial interactions, etc.). Future studies will be required to address these questions.

As production scales for engineered nanomaterials continue to expand, so too does the importance of developing strategies to minimize impurities and byproducts, and to improve overall nanomanufacturing efficiencies. In particular, some nanomanufacturing processes, such as arc-discharge synthesis of carbonaceous nanomaterials, require management strategies that prevent their release to the environment, either through treatment or recycling. Yields from this process are generally low; a recent study by Stevenson et al. (20), reported that 1000 g of feedstock were required to generate 1 g of final metallofullerene product,

with the remaining ~999 g of feedstock discarded as solid waste (or “waste soot”). On a mass basis, this solid waste fraction may be comprised of ~50% feedstock metals, some of which are commercially valuable (e.g., rare-earth metals) or toxic in environmental systems (e.g., Cu), and thus recent efforts have focused on recovering and recycling these materials (20, 34). This unexplored area of nanotechnology EHS poses significant challenges for public health officials, waste management authorities, and generators of nanomanufacturing wastes.

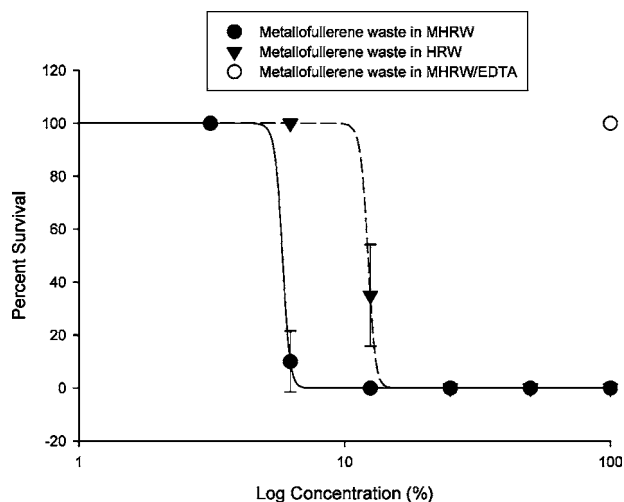
While it is imperative that researchers continue to evaluate the toxicological behavior of engineered nanomaterials, our results emphasize the need for studies to evaluate the occurrence and composition of impurities, particularly metals, in the products and byproducts of nanomanufacturing and waste streams. If present in sufficient concentrations, metals may confound experimental outcomes of studies examining the potential toxicity of engineered nanomaterials. This is particularly true in investigations where measurements and assays are sensitive to the presence of metals (e.g., tests with aquatic organisms such as fish and invertebrates). The implications of experimental outcomes are important both to ensure public health and safety and to communicate accurate information regarding the toxicological and fate/transport implications associated with engineered nanomaterials and associated waste byproducts. A recent study reported that the smaller sized fluorescent fraction of as-prepared single-walled nanotubes (SWNT) had greater impact on the estuarine copepod *Amphiascus tenuiremis* than purified SWNTs due to ingestion of carbonaceous nanomaterials and disruption of feeding, penetration of the gut wall, and/or oxidative stress (43). While this explanation is logical, the detection limits for metals by the EDX analysis used were not reported, and thus metals in the lower purity, as-prepared SWNTs cannot be eliminated as a potential contributor to toxicity to the metals sensitive test organism (44). While our study investigated wastes from different carbonaceous nanoparticles, our results imply that the metals in the tested, low-purity carbon nanomaterials were at adequate concentration to induce acute toxicity. Further, these results underscore the need to characterize test materials and media for the presence of impurities, and to consider toxicity mechanisms that may be attributable to these impurities.

Our results are not representative of all nanomaterial-based products and waste streams as there will likely be



**FIGURE 2.** Survivorship (48 h) in varying concentrations of metallofullerene waste and as-produced fullerene leachates for (a) *Ceriodaphnia dubia* and (b) *Pimephales promelas*. Leachates were prepared in moderately hard reconstituted water. To investigate the role of metals in toxicity of leachates, ethylenediaminetetraacetic acid (EDTA) was added to 100% leachate treatments—denoted by “(EDTA)” and open points. Means and standard deviations are plotted.

considerable variation among constituents present in nanomanufacturing outputs depending on the nature of the initial reactants and the efficiencies of the synthesis procedures used. Nevertheless, a global review of nanomanufacturing methods is warranted to evaluate the composition and management of waste streams from nanomanufacturing processes. Failure to consider the presence of metals and other nano as well as non-nano components in nanomanufacturing waste streams may lead to implementation of waste management strategies that do not account for the potential of these constituents to become mobilized once disposed of in the environment, such as through leaching from landfills or similar holding facilities (45, 46). Once specific waste materials are known, further investigation is required on a case-specific basis to determine the relevant concentrations of impurities released to the environment (e.g., the toxicity characteristic leaching procedure).



**FIGURE 3.** Survivorship (48 h) of *C. dubia* in varying concentrations (dilutions ranging from 3 to 100%) of metallofullerene waste leachate prepared in moderately hard reconstituted water (MHRW) versus hard reconstituted water (HRW). Means and standard deviations are plotted.

## Acknowledgments

Permission was granted by the Chief of Engineers to publish this information. The use of trade, product, or firm names in this paper is for descriptive purposes only and does not imply endorsement by the U.S. Government. The studies described and the resulting data presented herein were obtained from research supported by the Environmental Quality Technology Program of the U.S. Army Engineer Research and Development Center (Dr. John Cullinane, Technical Director). Funding for M. Hull was provided by a fellowship through the National Science Foundation Integrative Graduate Education and Research Traineeship (IG-ERT) program at Virginia Tech. Additional funding support was provided by a grant from the Virginia Tech Institute for Critical Technology and Applied Science (ICTAS) and NSF grant CBET-0537117. We thank H.C. Dorn of Virginia Tech for the generous donation of endohedral metallofullerene byproduct materials. We also thank Jennifer Goss and Archie Ball at ERDC for laboratory assistance.

## Supporting Information Available

Additional description is provided on the water column and sediment bioassays, statistical analysis, analytical chemistry, and leachate water quality. Also included is reporting on the prepared leachate water quality (Table S1), solid phase soot contaminants (Table S2), *Hyalella azteca* toxicity data (Table S3), schematic of the nanomanufacturing process and impurities (Figure S1), EDX plots of metals in the soot (Figure S2), metals concentrations of the metallofullerene leachate at varying ionic strengths (Figure S3), and metals concentrations in the as-produced fullerene leachate (Figure S4). This material is available free of charge via the Internet at <http://pubs.acs.org>.

## Literature Cited

- (1) Roco, M.; Bainbridge, W. Societal implications of nanoscience and nanotechnology: maximizing human benefit. *J. Nanopart. Res.* **2005**, *7*, 1–13.
- (2) Lux Research Inc. *The Nanotech Report: Investment Overview and Market Research for Nanotechnology*, 4th ed.; Lux Research: New York, NY, 2006; Vol. 1.
- (3) Son, S.; Bai, X.; Lee, S. Inorganic hollow nanoparticles and nanotubes in nanomedicine; Part 2: Imaging, diagnostic, and therapeutic applications. *Drug Discovery Today*. **2007**, *12*, 657–663.



- (4) Lekas D. Analysis of Nanotechnology from an Industrial Ecology Perspective Part II: Substance Flow Analysis Study of Carbon Nanotubes. Thesis submitted to Yale School of Forestry and Environmental Studies. 2005.
- (5) Jiang, L.; Gao, L.; Sun, J. Production of aqueous colloidal dispersions of carbon nanotubes. *J. Colloid Interface Sci.* **2003**, *260*, 89–94.
- (6) Ajayan, P.; Zhou, O. Applications of carbon nanotubes. In *Carbon Nanotubes: Synthesis, Structure, Properties and Application*; Dresselhaus M. S., Dresselhaus G., Avouris P. H., Eds.; Springer-Verlag: Berlin, Heidelberg, 2001; pp 391–425.
- (7) Hammond, P. Form and function in multilayer assembly: new applications at the nanoscale. *Adv. Mater.* **2004**, *16*, 1271–1293.
- (8) Defense Environment Alert. *Nanotechnology: EPA Crafting Nanotech Guide to Define 'New' Products Subject to TSCA*; U.S. Government Printing Office: Washington, DC, 2005; Vol. 13, p 1.
- (9) Nygren, K. Emerging technologies and the Army. *Advanced Materials and Process Technology Information Analysis Center (AMPTIAC) Quarterly Newsletter*. **2002**, *6*, 11–16.
- (10) Maynard, A.; Aitken, R.; Colvin, V.; Donaldson, K.; Oberdörster, G.; Philbert, M.; Ryan, J.; Seaton, A.; Stone, V.; Tinkle, S.; Tran, L.; Walker, N.; Warheit, D. Safe handling of nanotechnology. *Nature*. **2006**, *444*, 267–269.
- (11) Griffith, R.; Weil, R.; Hyndman, K.; Denslow, N.; Powers, K.; Taylor, D.; Barber, D. Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*). *Environ. Sci. Technol.* **2007**, *41*, 8178–8186.
- (12) Derfus, A.; Chan, W.; Bhatia, S. Probing the cytotoxicity of semiconductor quantum dots. *Nano Lett.* **2004**, *4*, 11–18.
- (13) Warheit, D.; Laurence, B.; Reed, K. Comparative pulmonary toxicity assessment of SWNTs in rats. *Toxicol. Sci.* **2004**, *77*, 117–125.
- (14) Helland, A.; Wick, P.; Koehler, A.; Schmid, K.; Som, C. Reviewing the environmental and human health knowledge base of carbon nanotubes. *Environ. Health Perspect.* **2007**, *115*, 1125–1131.
- (15) UK Health and Safety Executive (HSE). Health effects of particles produced for nanotechnologies. HSE Hazard assessment document EH75/6. 2004. available online at [www.hse.gov.uk/horizons/nanotech/healtheffects](http://www.hse.gov.uk/horizons/nanotech/healtheffects).
- (16) Lam, C.; James, J.; McCluskey, R.; Arepalli, S.; Hunter, R. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. *Crit. Rev. Toxicol.* **2006**, *36*, 189–217.
- (17) Maynard, A.; Aitken, R. Assessing exposure to airborne nanomaterials: Current abilities and future requirements. *Nanotoxicol* **2007**, *1*, 26–41.
- (18) Lecoanet, H.; Bottero, J.; Wiesner, M. Laboratory assessment of the mobility of nanomaterials in porous media. *Environ. Sci. Technol.* **2004**, *38*, 5164–5169.
- (19) Aitken, R.; Chaudhry, M.; Boxall, A.; Hull, M. Manufacture and use of nanomaterials: current status in the UK and global trends. *Occup. Med.* **2006**, *56*, 300–306.
- (20) Stevenson, S.; Coumbe, C.; Thompson, M.; Coumbe, L.; Phillips, J.; Buckley, J.; Wynne, J. Conversion of nanomaterial waste soot to recycled Sc<sub>2</sub>O<sub>3</sub> feedstock for the synthesis of metallic nitride fullerenes. *Ind. Eng. Chem. Res.* **2008**, *47*, 2096–2099.
- (21) Kiang, C.; VanLoosdrecht, P.; Beyers, R.; Salem, J.; Bethune, D.; Goddard, W.; Dorn, H.; Burbank, P.; Stevenson, S. Novel structures from arc-vaporized carbon and metals: Single-layer carbon nanotubes and metallofullerenes. *Surf. Rev. Lett.* **1996**, *3*, 765–769.
- (22) Kapustka, L. A.; Clements, W. H.; Ziccardi, L.; Paquin, P.; Sprenger, M.; Wall, D. Issue paper on the ecological effects of metals. In *Risk Assessment Forum*; U.S. Environmental Protection Agency: Washington, DC, **2004**.
- (23) Adams, W.; Chapman, P. Assessing the hazard of metals and inorganic metal substances in aquatic and terrestrial Systems. In *Proceedings from a Pellston Workshop*; SETAC Press: Pensacola, FL, 2007.
- (24) Belanger, S.; Farris, J.; Cherry, D. Effects of diet, water hardness, and population source on acute and chronic copper toxicity to *Ceriodaphnia dubia*. *Arch. Environ. Contam. Toxicol.* **1989**, *18*, 601–611.
- (25) Ahsanullah, M.; Negilski, D.; Mobley, M. Toxicity of zinc, cadmium and copper to the shrimp *Callinassa australiensis*. Effects of individual metals. *Mar. Biol.* **1981**, *64*, 299–304.
- (26) Witeska, M.; Jezierska, B. The effects of environmental factors on metal toxicity to fish (review). *Fresenius Environ. Bull.* **2003**, *12*, 824–829.
- (27) Stevenson, S.; Fowler, P.; Heine, T.; Duchamp, J.; Rice, G.; Glass, T.; Harich, K.; Hajdu, E.; Bible, R.; Dorn, H. Materials science—A stable non-classical metallofullerene family. *Nature (London)* **2000**, *408*, 427–428.
- (28) Guo, L.; Morris, D.; Liu, X.; Vaslet, C.; Hurt, R.; Kane, A. Iron bioavailability and redox activity in diverse carbon nanotubes samples. *Chem. Mater.* **2007**, *19*, 3472–3478.
- (29) Stevenson, S.; Mackey, M.; Thompson, M. Effect of copper metal on the yield of Sc<sub>3</sub>N@C-80 metallofullerenes. *Chem. Comm.* **2007**, *41*, 4263–4265.
- (30) Tian, Y.; Zhang, Y.; Yu, Q.; Wang, X.; Hu, Z.; Zhang, Y.; Xie, K. Effect of catalysis on coal to nanotube in thermal plasma. *Catal. Today*. **2004**, *89*, 233–236.
- (31) Asokan, S.; Krueger, K.; Alkhalwaldeh, A. The use of heat transfer fluids in the synthesis of high-quality CdSe quantum dots, core/shell quantum dots, and quantum rods. *Nanotechnol.* **2005**, *16*, 2000–2011.
- (32) Pumera, M. Carbon nanotubes contain residual metal catalyst nanoparticles even after washing with nitric acid at elevated temperature because these metal nanoparticles are sheathed by several graphene sheets. *Langmuir*. **2007**, *23*, 6453–6458.
- (33) Stevenson, S.; Rice, G.; Glass, T.; Harich, K.; Cromer, F.; Jordan, M.; Craft, J.; Hadju, E.; Bible, R.; Olmstead, M.; Maitra, K.; Fisher, A.; Balch, A.; Dorn, H. Small-bandgap endohedral metallofullerenes in high yield and purity. *Nature*. **1999**, *401*, 55–57.
- (34) Hull, M. Alternative products from nanomanufacturing wastes. National Science Foundation Phase I Small Business Innovation Research Grant. Award No. 0637924. Available online at <http://nsf.gov/awardsearch/showAward.do?AwardNumber=0637924>.
- (35) *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, EPA/812/R/02/012; U.S. Environmental Protection Agency, Office of Water: Washington, D.C., 2002.
- (36) Hockett, J.; Mount, D. Use of metal chelating agents to differentiate among sources of acute aquatic toxicity. *Environ. Toxicol. Chem.* **1996**, *15*, 1687–1693.
- (37) Chen, W.; Duan, L.; Zhu, D. Adsorption of polar and nonpolar organic chemicals to carbon nanotubes. *Environ. Sci. Technol.* **2007**, *41*, 8295–8300.
- (38) Saha, B.; Tai, M.; Streat, M. Metal sorption performance of an activated carbon after oxidation and subsequent treatment. *Process Saf. Environ. Prot.* **2001**, *79*, 345–351.
- (39) Stumm, W.; Morgan, J. *Aquatic Chemistry, Chemical Equilibria and Rates in Natural Waters*, 3rd ed.; John Wiley & Sons, Inc: New York, NY, 1996.
- (40) Powell, J. Separation chemistry. In *Handbook on the Physics and Chemistry of Rare Earths*; Gschneider, K., Eyring, L., Eds.; North-Holland Publishing: Amsterdam, 1979; *8*, pp 1–109.
- (41) *National Recommended Water Quality Criteria*, EPA/822/R/02/047; U.S. Environmental Protection Agency, Office of Water: Washington, D.C., 2002.
- (42) Carlson, A.; Nelson, H.; Hammermeister, D. Development and validation of site-specific water-quality criteria for copper. *Environ. Toxicol. Chem.* **1986**, *5*, 997–1012.
- (43) Templeton, R.; Ferguson, P.; Washburn, K. Life-cycle effects of single-walled carbon nanotubes (SWNTs) on an estuarine meiobenthic copepod. *Environ. Sci. Technol.* **2006**, *40*, 7387–7393.
- (44) Hagopian-Schlekat, T.; Chandler, G.; Shaw, T. Acute toxicity of five sediment-associated metals, individually and in a mixture, to the estuarine meiobenthic harpacticoid copepod *Amphiascus tenuiremis*. *Mar. Environ. Res.* **2001**, *51*, 247–264.
- (45) Ward, M.; Bitton, G.; Townsend, T.; Booth, M. Determining toxicity of leachates from Florida municipal solid waste landfills using a battery-of-tests approach. *Environ. Toxicol. Chem.* **2002**, *17*, 258–266.
- (46) Osaki, K.; Kashiwada, S.; Tatarazako, N.; Ono, Y. Toxicity testing of leachate from waste landfills using medaka (*Oryzias latipes*) for monitoring environmental safety. *Environ. Monit. Assess.* **2006**, *117*, 73–84.

ES802483P